A facile optosensing protocol based on molecularly imprinted polymer coated on CdTe quantum dots for highly sensitive and selective amoxicillin detection

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Abstract

A facile method for coating a molecularly imprinted polymer onto CdTe quantum dots (MIP-QDs) was successfully formulated and for the first time used as a highly selective and sensitive fluorescence probe for the determination of trace amoxicillin. The MIP-QDs was prepared using a sol-gel process with 3-aminopropylethoxysilane as a functional monomer, tetraethoxysilane as a cross-linker and amoxicillin as a template molecule. After removal of the template molecule from polymer layer, MIP-QDs containing cavities specific to amoxicillin were obtained. The hydrogen bonding between the amino group of 3-aminopropylethoxysilane and functional groups of amoxicillin and the specific size and shape of the cavity provided good selectivity. The fluorescence intensity of MIP-QDs was more strongly quenched by amoxicillin compared to a non-imprinted polymer (NIP-QDs) with an imprinting factor of 43.6. Under optimum condition, the fluorescence intensity of MIP-QDs was decreased in response to increasing the amoxicillin concentration with good linearity in the range of 0.20 to 50.0 μg L^{-1}. The limit of detection and the limit of quantitation were 0.14μg L^{-1} and 0.46 μg L^{-1}, respectively. The developed method showed good repeatability and reproducibility with the relative standard deviation being less than 6 %. This developed method was successfully applied for the determination of amoxicillin in egg, milk and honey samples with a satisfactory recovery of 85 – 102% being achieved.

Keyword: CdTe quantum dot, molecularly imprinted polymer, amoxicillin, fluorescence probe

1. Introduction

Amoxicillin is a synthetic antibiotic in the β-lactam class of antibiotics and is extensively used to treat infectious diseases in human and animal, being active against both Gram-positive and Gram-negative bacteria [1-3] due to its broad spectrum antibiotic activity and low cost [4]. However, the usage of antibiotic in food-producing animals leading to the presence of residues in food and the environment[4] which can cause some side-effects such as hypersensitivity in humans [5]. To assure the consumers safety, the European Union (EU) has set a maximum residue limit (MRL) of amoxicillin of 50 $\mu g\ kg^{-1}$ in animal tissues and 4.0 $\mu g\ kg^{-1}$ in milk [6]. Therefore, the monitoring of amoxicillin in food products is an important application. Various analytical methods have been developed for the determination of amoxicillin such as chromatography [4,7,8], electrochemical methods [2,3,9], surface plasmon resonance [10] and spectrophotometry [1,11-13]. Among these methods, fluorescence spectroscopy is an interesting alternative method because it has a short analysis time, is relatively simple to use, low cost of equipment, requires small sample amounts and minimal consumption of organic solvents [12,14]. Several organic dyes have been used as a fluorescence probes to detect various target analytes [15], however they often have some drawbacks such as broad emission bands and mostly asymmetric spectra [16]. To overcome this problems, quantum dots (QDs) have attracted much attention in recent years for use as fluorescence probes for the determination of ions, molecules, proteins and cells [17-20] due to their desirable optical properties such as size-dependent emission, narrow symmetric emission bands, long fluorescence lifetime, photochemical stability and good water dispersibility[21,22]. To improve the selectively of this method the surface of QDs need to be modified with some specific materials [18]. Molecularly imprinted polymers (MIPs) are an attractive strategy to modify the surface of quantum dots. MIP

are affinity polymers and can be synthesized to be specific to a target molecule, they are easy to prepare, inexpensive and display good stability [23]. These materials are highly cross-linked three-dimensional network polymers, formed by polymerization between a functional monomer and a cross-linking monomer including template molecules (target analytes) [24,25]. After the polymerizations were complete, the template molecules were removed and the specific cavities were obtained [26]. MIPs combined with QDs have been developed as a fluorescence probe for a selective determination of some compounds i.e. histamine [27], malachite green [28], chlorpyritos [29] and α-fetoprotein [30].

In this work, molecularly imprinted polymers coated on quantum dots (MIP-QDs) were synthesized and used as a fluorescence probe for the highly sensitive and selective determination of amoxicillin. The fluorescence properties and morphology of the synthesized MIP-QDs were investigated and characterized. The effects of various parameters on the analytical performance were also optimized. The developed MIP-QDs were applied for the determination of amoxicillin in egg, milk and honey samples. The developed method was compared with a HPLC method and the recovery from these samples was also investigated.

2. Experimental

2.1 Chemicals and reagents

All chemicals were of analytical grade, amoxicillin, 3-Aminopropyl triethoxysilane (APTES, ≥ 98%), tetraethyl orthosilicate (TEOS, ≥ 99%), tellurium powder (99.8%), thioglycolic acid (TGA) and sodium borohydride ($NaBH_4$) were purchased form Sigma-Aldrich (St. Louis, MO, USA). $CdCl_2.2H_2O$ was purchased from Asia Pacific Specialty Chemicals Co. Ltd. (NSW, Australia). Tris (hydroxymethyl) aminomethane and ethanol (≥ 98%) were purchased from Merck (Frankfurt, Germany). Ammonia and sodium hydroxide were purchased

from RCI Labscan(Bangkok, Thailand). Ultrapure water was from a water purification system (18.2 MΩ.cm^{-1}) (Elgastat Maxima, ELGA, UK)

2.2 Instrumental

Fluorescence spectroscopy was performed using a Shimadzu RF-5301PC spectrofluorophotometer (Kyoto, Japan). UV/Vis absorption spectra were recorded using an Avaspec 2048 spectrometer (Avantes, Apeldoorn, Netherlands). The morphologies of MIP-QDs and NIP-QDs were obtained using a scanning electron microscope (JSM–5200, JEOL, Tokyo, Japan) and the FTIR spectra were recorded using FTIR spectroscopy (PerkinElmer, Waltham, MA, USA). TEM images were obtained from a JEM-2010 transmission electron microscope (JEOL, Japan). BET surface areas of MIP-QDs and NIP-QDs were determined using QuantachromeAutosorb 1 system (Quantachrome Instruments, USA).

2.3 Synthesis of TGA- capped CdTe QDs

TGA-capped CdTe quantum dots were synthesized using a method modified from previous work [17, 18]. Firstly,50 mg of tellurium powder and 40 mg of $NaBH_4$ were dissolved in 1.0 mL of deionized water under a nitrogen atmosphere to prepare a NaHTe solution. Also, 0.050 g of $CdCl_2$ and 25.0 μL TGA were dissolved in 100.0 mL of deionized water and adjusted to pH 11.50 with 1.0 M NaOH. Then, this solution mixture was placed into a three-necked flask anddeaerated by bubbling with nitrogen gas for 15 min. Under vigorous stirring, 500 μL of NaHTe solution was rapidly injected into the solution mixture under a nitrogen atmosphere. Then the solution was refluxed for 10 min at 90 °C. The resulting product was precipitated with ethanol to remove excess reagents and centrifuged at 2800 RCF for 10 min. The obtained TGA-capped CdTe QDs were dried in an oven at 40 °C for 1 hand kept in a desiccator for further use.

2.4 Synthesis of molecularly imprinted polymer coated CdTe quantum dots (MIP-QDs)

Briefly, 6.6 mgof amoxicillin (template) was dissolved in 5.0 mL of deionized water and mixed with 30 μL APTES (functional monomer) in a brown bottle and stirred at room temperature (25°C) for 1 h. Then, 15 mL of TGA-capped CdTe QDs (7.5×10^{-5} M), 135μL of TEOS (cross-linker) and 150 μL of 25% ammonia solution were added and continuously stirred for 6 h. The resultant products were centrifuged and washed three times with 10 mL of ethanol to remove template molecules. Finally, the MIP-QDs were dried in an oven at 40 °C for 1 h. The non-imprinted polymer coated QDs (NIP-QDs) were also prepared through the same procedure but without addition of the template molecule.

2.5Fluorescence measurements

Fluorescence measurements were performed using a spectra band pass of the excitation and emission of 10 nm, an excitation wavelength of 355 nm and recording the emission in the range of 400 – 700 nm. The measurements were carried by mixing of 150 μL of MIP-QDs or NIP-QDs solution with 50 μL of amoxicillin solution. All measurements were performed at room temperature (25 °C) for convenient analysis.

2.6Sample preparation of food samples

All samples were purchased from the local market in Hat Yai, Songkhla, Thailand. Milk samples were pretreated according to the previous report [31]. Briefly, 30 mL of raw milk was transferred to a 50 mL polypropylene centrifuge tube and centrifuged at 1680 RCF for 15 min to precipitate fat. Then 10 mL of acetonitrile was added into the defatted milk to deproteinize it. Subsequently, the mixture was vortexed and centrifuged at 2240 RCF for 15 min. Then, the supernatant was collected and evaporated to dryness at 40 °C. The extract was redissolved with 2.0 mL of phosphate buffer and analysed by the developed method. For honey samples, the

sample preparation was modified from previous work [3]. Briefly, 10 mL of honey was transferred to a 50 mL polypropylene centrifuge tube and diluted with 10 mL of distilled water. The mixture was then vortexed for 1 min followed by centrifuging at 2240 RCF for 20 min and the supernatant was evaporated at 60 °C. Then, 2.0 mL of phosphate buffer was added and vigorously vortexed for 10 s before analysis. The preparation of egg sample was modified from previous work [4], whole egg white was homogenized and 5.0 g of homogenized egg was transferred into a 50 mL polypropylene centrifuge tube and 10 mL of acetonitrile was added. The mixture was extracted by ultrasonification for 15 min and then centrifugation at 2240 RCF for 10 min, the supernatant was transferred to another 50 mL polypropylene centrifuge tube. Then 1.0 mL of ammonium acetate buffer was added and mixed by vortexing. 20 mL of dichloromethane was added into the mixture and vortexed for 1 min and centrifuged at 2240 RCF for 10 min; the supernatant was collected and evaporated at 40 °C. The extractant was redissolved with 2.0 mL of phosphate buffer and filtered through a 0.22 μm membrane filter before analysis.

3. Results and discussion

3.1 The synthesis of MIP coated on TGA-capped CdTe quantum dots (MIP-QDs)

The MIP-QDs were synthesized as shown in **Fig. 1.** TGA-capped CdTe QDs has a carboxylic group which can interact with TEOS and APTES to form a sol-gel MIP coated onto QDs. The strong non-covalent interaction between APTES and amoxicillin occurred during the molecular imprinting process. The amino group of APTES can interact with functional groups of amoxicillin such as the carboxylic group, amino group and hydroxyl group though hydrogen bonding. The synthesized MIP-QDs and NIP-QDs have a symmetric emission at 545 nm when excited at 355 nm.

The fluorescence intensity of MIP-coated QDs before the removal of templates molecules was relatively weak **(Fig. 2a)**. However, the fluorescence intensity of MIP-QDs was restored dramatically after removal of template molecules**(Fig. 2b)**. The fluorescence intensity was restored to almost the same value as obtained with NIP-QDs **(Fig. 2c)**. These results indicated that the template molecules were almost completely removed from the recognition cavities in the MIP-QDs. In addition, the fluorescence signal was sharp which indicated that the size of MIP-QDs were homogeneous. **Fig. 2d** and **Fig. 2e** showed the fluorescence photographs of MIP-QDs without and with amoxicillin.

3.2 Characterizations of MIP-QDs

The fluorescence spectrum and absorption spectrum of TGA-capped CdTe QDs are shown in **Fig. S1**. The TGA-capped CdTe QDs showed a narrow and symmetric fluorescence spectrum. The maximum absorption wavelength of the TGA-capped CdTe QDs at 500 nm was used to calculate the particle size of QDs as described in previous work [18], the average particles size was approximately 2.5 nm. The TEM images of the TGA-capped CdTe QDs and MIP-coated CdTe QDs are shown in **Fig. 3A** and **3B**. The TEM image exhibits the uniform size and distribution of TGA-capped CdTe and their particles size agreed with those calculated.

The morphology of MIP-QDs was investigated by SEM. As shown in **Fig. 3C**, they have a uniform spherical shape and narrow size distribution with diameters in the range of 180 - 200 nm. It is indicated that the particles diameter increased significantly after coating with MIP compared with original TGA-capped CdTe QDs. This indicated that the MIP-QDs have a large surface area with effective imprinting sites to bind the template molecule.

The FT-IR spectrum of TGA capped CdTe QDs **(Fig. 4a)** showed a characteristic peak at 1375 and 1572 cm^{-1} which corresponded to the C=O stretching and deformation vibration of

carboxylic group. The absorption peaks at 3400 and 1225 cm^{-1} were attributed to the O-H stretching and C-O stretching. The FT-IR spectrum of amoxicillin **(Fig. 4b)** exhibited an absorption band at 3463 cm^{-1} corresponding to the N-H stretching of primary amine. The absorption band at 1777 cm^{-1} and 1687 cm^{-1} corresponded to the C=O stretching of carbonyl and carboxylic group, respectively. The absorption peak at 1590 cm^{-1} was due to the C-C stretching of the thiazole ring. The FT-IR spectrum of MIP-QDs before removal of the template (amoxicillin) is shown in **Fig. 4c**. The absorption peak at 1065 cm^{-1} was ascribed to Si-O-Si asymmetric stretching. The Si-O vibrations band was shown at 460 and 784 cm^{-1}. After removing the template the absorption peak at 1777, 1687 and 1590 cm^{-1}which related to amoxicillin were absent **(Fig. 4d)**. The results indicated that the molecularly imprinted polymer was successfully synthesized and coated on the CdTe-QDs to form MIP-QDs for selective recognition for amoxicillin.

The photoluminescence quantum yields of the TGA-capped CdTe QDs and MIP-coated QDs were 0.67 and 0.30, respectively.The BET surface area of MIP-QDs and NIP-QDs were 12.00 ± 0.18 m^2 g^{-1} and 11.50 ± 0.15 m^2 g^{-1}, respectively. The MIP-QDs showed higher surface area than NIP-QDs, this results from the imprinted cavity of the template (amoxicillin).

3.3 Optimization of the determination conditions

To obtain the highest sensitivity and shortest analysis time, the ratio of amoxicillin solution to MIP-QDs solution,molar ratio of template to monomer, incubation time and pH value were investigated and optimized. The sensitivity was obtained by the determination of amoxicillin in the concentration range of 0.20 to 50.0 μg L^{-1}. All optimization parameters were performed at room temperature (25 °C) for convenient analysis.

3.3.1 The ratio of amoxicillin solution to MIP-QDs solution

The different ratios of amoxicillin solution to MIP-QDs solution (80 μg L^{-1}) was investigated for the determination of amoxicillin *i.e.*1:1, 1:2, 1:3 and 1:4. The results are shown in **Fig. 5A**, the sensitivity increased when the ratio of MIP-QDs increased up to ratio of 1:3 and after that no further increase in sensitivity at higher ratio. Therefore, the ratio of amoxicillin solution to MIP-QDs solution of 1:3 was chosen for further studies.

3.3.2 Effect of incubation time

To ensure the completion binding between amoxicillin and the recognition site of MIP-QDs, the effect of incubation time between amoxicillin and MIP-QDs was investigated from 0 to 60 min. As shown in **Fig. 5B**, the fluorescence intensity change (F0/F) of MIP-QDs increased up to an incubation time of 30 min and above that remains almost constant. Therefore, an incubation time of 30 min was selected for further experiments. Although, the incubation time between MIP-QDs and amoxicillin was 30 min, however, the measurement of the fluorescence intensity requires only 2minutes. This indicated that the developed method is capable of a high throughput of approximately 30 samples per 1 h.

3.3.3 Ratio of template to monomer

To obtain the highest the sensitivity of MIP-QDs for the determination of amoxicillin, the effect of molar ratio of template to monomer was investigated and the results are shown in **Fig. 5C.**The highest sensitivity was obtained at the molar ratio of 1:8. The molar ratio of template to monomer of 1:6 provided lower sensitivity due to its low number of recognition sites for the target analytes. While, the sensitivity was also decreased at the molar ratio of 1:10 due to the non-forming excess monomer might inhibit the binding of recognition sites and the target analytes. It resulted in the decreasing of sensitivity.

3.3.4 Effect of pH

MIP-QDs are sensitive to their surrounding environment and the pH value had a significant effect on the fluorescence intensity of MIP-QDs [32]. Extremes of pH value both high and low will affect the binding efficiency of MIP-QDs and target analytes [33]. Therefore, the influence of the pH of buffer solution (Tris-HCl) used to prepare MIP-QDs solution in the range of 6.0–10.0 were investigated. The results are showed in **Fig. 5D**, the pH value had a significant influence on the fluorescence quenching of MIP-QDs with the highest sensitivity being obtained at pH 8.0. The sensitivity decreased at lower pH due to hydrogen ion in the solution affecting the hydrogen bonding between MIP-QDs and amoxicillin [34] and the resulting of the protonation of theamine groups of amoxicillin.At a pH higher than 8.0, the sensitivity was also decreased, possibly because of degradation of the template molecule [34, 35]. The pK_a value of amoxicillin was 2.4, 7.4 and 9.6 [36]. In addition, the silica shell was ionized under highly alkaline condition which can cause damage to the structure of the binding site.As a result, a Tris-HCl buffer solution pH of 8.0 was selected as an optimum value and used for the further experiment.

3.4 Comparison between MIP-QDs and NIP-QDs for the determination of amoxicillin

To investigate the recognition ability of the MIP-QDs compared to NIP-QDs, the fluorescence intensity of MIP-QDs and NIP-QDs with different concentration of amoxicillin were investigated. As shown in **Fig. 6A,**the fluorescence intensity of MIP-QDs clearly decreased with increasing concentration of amoxicillin whereas the fluorescence intensity of NIP-QDs does not significantly change **(Fig. 6B).**It can be seen in **Fig. 6C**; the fluorescence intensity is proportionally quenched much more for MIP-QDs than for NIP-QDs. The higher quenching efficiency or better sensitivity of MIP-QDs results from their specific binding affinity towards

amoxicillin due to the cavities in MIP-QDs. These results indicated the synthesized MIP-QDs could be used as a facile and effective method to detect amoxicillin.

The fluorescence quenching of the procedure could be described by the Stern-Volmer equation[27-30]:

$$F_0/F = 1+ K_{sv}[C] \quad (1)$$

Where F_0 and F are the fluorescence intensity of MIP-QDs in the absence and presence of a given concentration of amoxicillin, respectively. K_{sv} is the Stern-Volmer constant and [C] is the concentration of quencher (amoxicillin). The equation was used to quantify quenching constant of the MIPs and NIPs. The imprinting factor (IF), which is the ratio of K_{sv} of the MIP-QDs and NIP-QDs (IF = K_{sv},MIP/K_{sv},NIP) was used to evaluate the selectivity of sensing materials. Under optimum conditions, the imprinting factor was calculated to be 43.6which indicates the specific cavities of MIP are capable of selective adsorption of amoxicillin leading as demonstrated by greatly increased fluorescence quenching efficiency.

3.5 Selectivity of MIP-QDs for the determination of amoxicillin

The selectivity of the developed MIP-QDs was investigated by comparing the fluorescence intensity of MIP-QDs after interaction with the target analyte (amoxicillin) and other antibiotics. The potential interferent antibiotics used were ampicillin (AMP), cephalexin (CEP), penicillin G (PEG), chloramphenicol (CRP) and thiamphenicol (TAP).The fluorescence intensity of MIP-QDs showed a high response to amoxicillin and a much lower signal for other antibiotics. The Stern-Volmer constant (K_{sv}) values of amoxicillin interaction with MIP-QDs were higher than other antibiotics as shown in **Fig. 7**. These results indicated the MIP-QDs have a good selectivity for amoxicillin detection. This can be explained by the synthesis process of

MIP-QDs producing many specific recognition sites with respect to the amoxicillin on the surface of MIP-QDs, the analyte therefore could be bound strongly to the MIP-QDs leading to quenching of the fluorescence intensity.

3.6 Stability of MIP-QDs

The stability of the synthesized MIP-QDs in a Tris-HCl buffer solution (pH 8.0) was investigated at room temperature (25 °C) by the repeated measurement of the fluorescence intensity over time. As shown in **Fig. S2,** the fluorescence intensity decreased less than 1.0 % after 12 h. The stability of the solid powder of MIP-QDs was also investigated by keeping it in a desiccator at 25 °C and the fluorescence intensity showed no significant changes after 3 months **(Fig. S3)**. These results indicated that MIP-QDs had good stability since coating the CdTe QDs with an MIP outside layer helps to improve the QDs photo stability.

3.7 Analytical performance of MIP-QDs for the determination of amoxicillin

The analytical performance of the developed method was evaluated including linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility. The MIP-QDs exhibited linear fluorescence quenching for amoxicillin detection in the concentration range from 0.20 to 50.0μg L^{-1} with a coefficient of determination (R^2) of 0.9994 as shown in**Fig. 8.** The LOD and LOQ were 0.14 μg L^{-1} and 0.46 μg L^{-1}, respectively, based on IUPAC criteria, 3σ/k and 10σ/k, respectively., where σ is the standard deviation of the blank measurement (n = 20) and k is the slope of the calibration curve.

Five replicate measurements of amoxicillin (50 μg L^{-1}) were performed to evaluate the precision of the MIP-QDs measurement. Under the optimum conditions, the relative standard deviation was 2.7 %, indicating good measurement repeatability.

The reproducibility of synthesized MIP-QDs was investigated by preparing six different batches of MIP-QDs under identical conditions. The synthesized MIP-QDs were used to determine amoxicillin at a concentration of 50 μg L^{-1}under optimum condition. The relative standard deviation (RSD) was 5.0 %, indicating good MIP-QDs preparation reproducibility.

3.8 Application of MIP-QDs for the determination of amoxicillin in food samples

The developed method using MIP-QDs as fluorescence probes was applied to determine amoxicillin in a range of food samples i.e. egg, milk and honey. The concentration of amoxicillin was found in milk sample as supplied at a concentration of 0.50 μg L^{-1}. The accuracy of the developed method was also investigated by spiking the food samples with amoxicillin at four different concentrations (1.0, 10, 20, 50 μg L^{-1}).The samples preparation was described in section 2.5. The amoxicillin recovery values of spiked samples were obtained in the range of 85.3 to 102.0% with a relative standard deviation less than 6 % (**Table 1).** The result showed that MIP-QDs can be used in the determination of amoxicillin in food samples with good accuracy and precision.

The developed MIP-QDs method was compared with a HPLC method. The three different samples (egg, milk, honey) were spiked with the four different concentrations of amoxicillin. The extracted sample solutions were analyzed using both MIP-QDs and HPLC method. The typical HPLC chromatogram of spiked egg samples as shown in **Fig. 9A.** The correlation between both methods was very good as shown in **Fig. 9B**, the coefficient of determination (R^2) was 0.9939. The result indicated that the developed method agrees well with the HPLC method and can be used for the determination of trace amoxicillin in food samples.

3.9 Comparison of the developed MIP-QDs method with other methods

The analytical performances of the developed MIP-QDs was compared with other works for the determination of amoxicillin and summarized in **Table 2.** The developed method provided a wide linear range and the limit of detection was much lower than other methods [3,13,37-42], while the recovery and precision of this method were comparable or better than other methods. Moreover, when compared this method with chromatographic methods [37-39], the fluorescence measurement is simpler, faster, cost-effective and did not require any organic solvents such as normally used in the HPLC mobile phase. In addition, the selectivity of this work was improved with the use of MIPs and sensitivity was improved with quantum dots. Therefore, the developed MIP-QDs method can be used as an effective method for a simple, rapid, cost-effective, sensitive and selective determination of amoxicillin in food samples.

4. Conclusion

The facile synthesis of a molecularly imprinted polymer coated onto CdTe quantum dots was successfully achieved and the resultant composite used as a highly sensitive and selective fluorescence probe for the determination of amoxicillin. The detection is based on the fluorescence quenching of the MIP-QDs after the binding of amoxicillin into the specific cavities of MIP-QDs. The MIP-QDs fluorescence probe combined the advantages of the high sensitivity of QDs and good selectivity of the MIP, demonstrating highly sensitivity allowing detection of amoxicillin at trace levels and good selectivity to amoxicillin based on shape, size and functional group interactions. The developed MIP coated CdTe QDs was successfully applied to the determination of amoxicillin in various complex sample matrices *i.e.* eggs, milk and honey. The advantages of this method include simplicity, rapidity, high sensitivity, good selectivity and cost efficiency.

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Table 1. Application of MIP-QDs and analytical results for the determination of amoxicillin in food samples (*n*=3)

Samples	Amoxicillin (μg L^{-1})		Recovery (%)	RSD
	A mount added	Amount found		
Egg 1	0.0	n.d.	-	-
	1.0	0.86	86.0	1.3
	10.0	8.79	87.9	3.6
	20.0	18.79	94.0	3.1
	50.0	45.81	91.6	1.3
Egg 2	0.0	n.d.	-	-
	1.0	0.85	85.3	3.3
	10.0	8.63	86.3	4.6
	20.0	18.81	94.1	3.5
	50.0	46.75	93.5	2.0
Milk 1	0.0	n.d.	-	-
	1.0	1.02	102.0	2.7
	10.0	9.75	97.4	2.2
	20.0	19.53	97.7	4.6
	50.0	46.12	92.2	1.8
Milk 2	0.0	0.50	-	2.0
	1.0	1.48	97.8	5.7
	10.0	9.97	94.7	3.9
	20.0	19.62	95.6	2.4
	50.0	47.0	93.0	2.3
Honey 1	0.0	n.d.	-	-
	1.0	0.86	86.0	1.7
	10.0	8.91	89.1	3.7
	20.0	18.41	92.1	2.2
	50.0	43.57	87.1	3.6
Honey 2	0.0	n.d.	-	-
	1.0	0.99	98.5	3.1
	10.0	8.72	87.2	3.5
	20.0	18.12	90.6	4.2
	50.0	48.43	96.9	4.8

n.d. is not detectable

Table 2 Comparison of the developed MIP-QDs method with other methods for the determination of amoxicillin

Detection technique	Samples	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)	References
MIP grown on MWCNTs/electrochemical	Milk and Honey	36.5-2190.0	32.5	88-96	1.8-3.8	[3]
SPME/HPLC-UV	Plasma	1,000-50,000	1210	-	5.9	[37]
HFCU/HPLC/UV	Human Plasma	100-20,000	25	83.1-86.8	3.1	[38]
SPE/HPLC-UV	Milk and river water	2-500	1.5	93-103	11	[39]
Polyglutamic acid modified glassy carbon electrode/ electrochemical	Human urine	730.8-9135.0	336.2	96.2-106.7	1.4-2.5	[40]
SPE/spectrophotometric	Pharmaceutical formulations, human urine	5-1000	3.0	96.2-102.1	0.7-1.3	[41]
FIA/spectrophotometric	Pharmaceutical preparation samples	2,000-40,000	637	97-102	0.4-1.5	[13]
Colorimetric	Capsules and oral suspension	110-1644	54.8	-	2.1	[42]
MIP-QDs	Egg, Milk, Honey	0.2-50	0.14	85-102	1.3-5.7	This work

SPME=Solid phase microextraction

SPE=Solid phase extraction

HFCU=Hollow fiber centrifugal ultrafiltration

MIP=Molecularly imprinted polymer

MWCNTs=Multiwalled carbon nanotubes

FIA=Flow injection analysis

Figure Captions

Fig. 1 The synthesis process of MIP-QDs for the recognition of amoxicillin.

Fig. 2 Fluorescence spectra of MIP-CdTe QDs before removal of template molecules (a) and after removal of template molecules (b), NIP-CdTe QDs (c). The fluorescence photographs of MIP-CdTe QDs (d) and MIP-CdTe QDs + amoxicillin(e).

Fig. 3 TEM images of TGA-capped CdTe QDs (A), MIP-QDs (B) and SEM image of MIP-QDs (C).

Fig. 4 FTIR spectra of (a) TGA capped CdTe QDs**,** (b) amoxicillin, (c) MIP-QDs before removal of the template and (d) MIP-QDs after removal of the template.

Fig. 5 (A) The effect of ratio of amoxicillin solution to MIP-QDs solution, (B) incubation time, (C) molar ratio of template to monomer and (D) pH value on the fluorescence quenching of MIP-QDs for the determination of amoxicillin.

Fig. 6 Fluorescence spectra of MIP-QDs (A), NIP-QDs (B) and the calibration curve (C) in the presence of amoxicillin in the concentration range of 0.0 to 50.0 $\mu g\ L^{-1}$.

Fig. 7 Selectivity of MIP-QDs, Stern-Volmer constant for fluorescence quenching of MIP-QDs for amoxicillin and other antibiotics (results are mean of three replicates).

Fig. 8 Fluorescence spectra of MIP-QDs at different concentration of amoxicillin and (inset) calibration curve, (results are mean of three replicates).

Fig.9 (A) HPLC chromatogram of spiked egg sample at different concentration of amoxicillin; (a) 0.10 mg L^{-1}, (b) 1.0 mg L^{-1}, (c) 2.0 mg $L^{-1}$and (d) 5.0 mg L^{-1},(B) Correlation between MIP-QDs and HPLC methods for the determination of amoxicillin in food samples

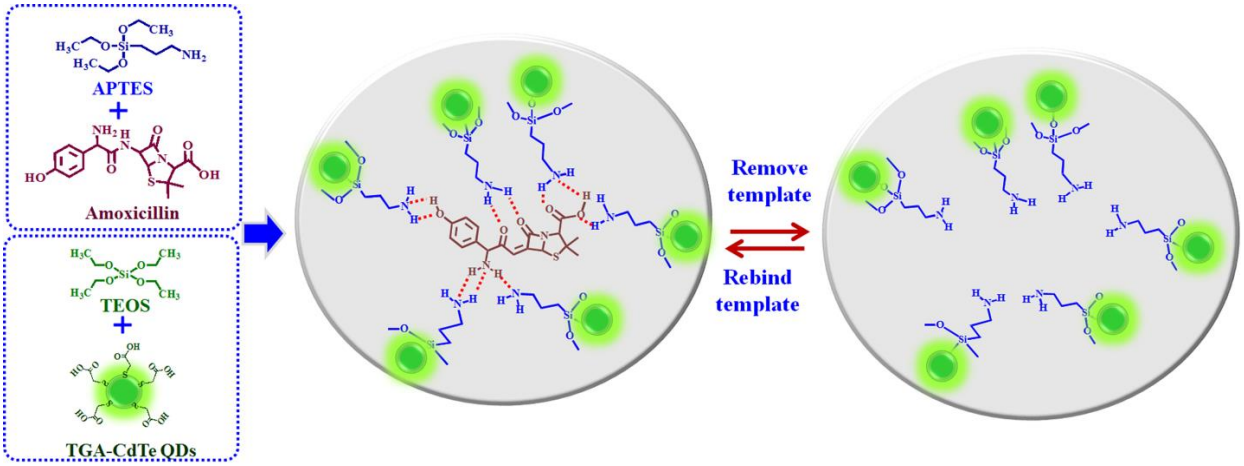

APTES
Amoxicillin
TEOS
TGA-CdTe QDs
Remove template
Rebind template

Fig. 1

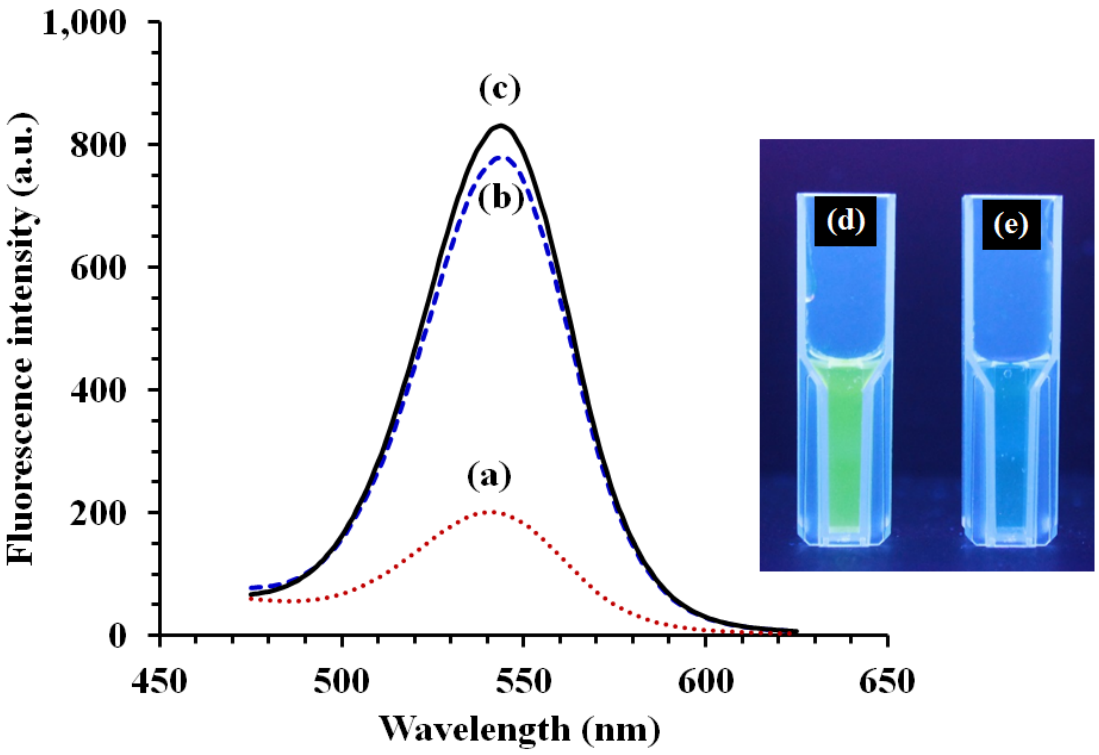

1,000
800
600
400
200
0
Fluorescence intensity (a.u.)
450
500
550
600
650
Wavelength (nm)
(a)
(b)
(c)
(d)
(e)

Fig. 2

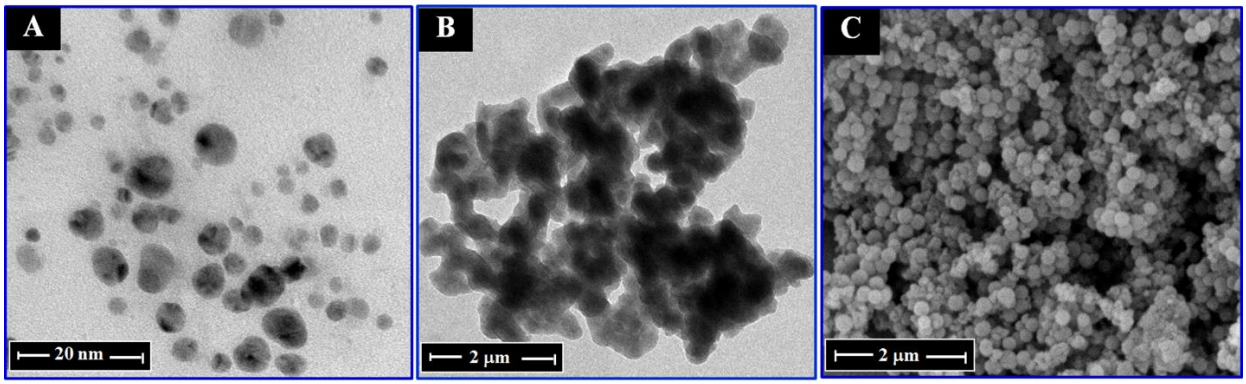


Fig. 3

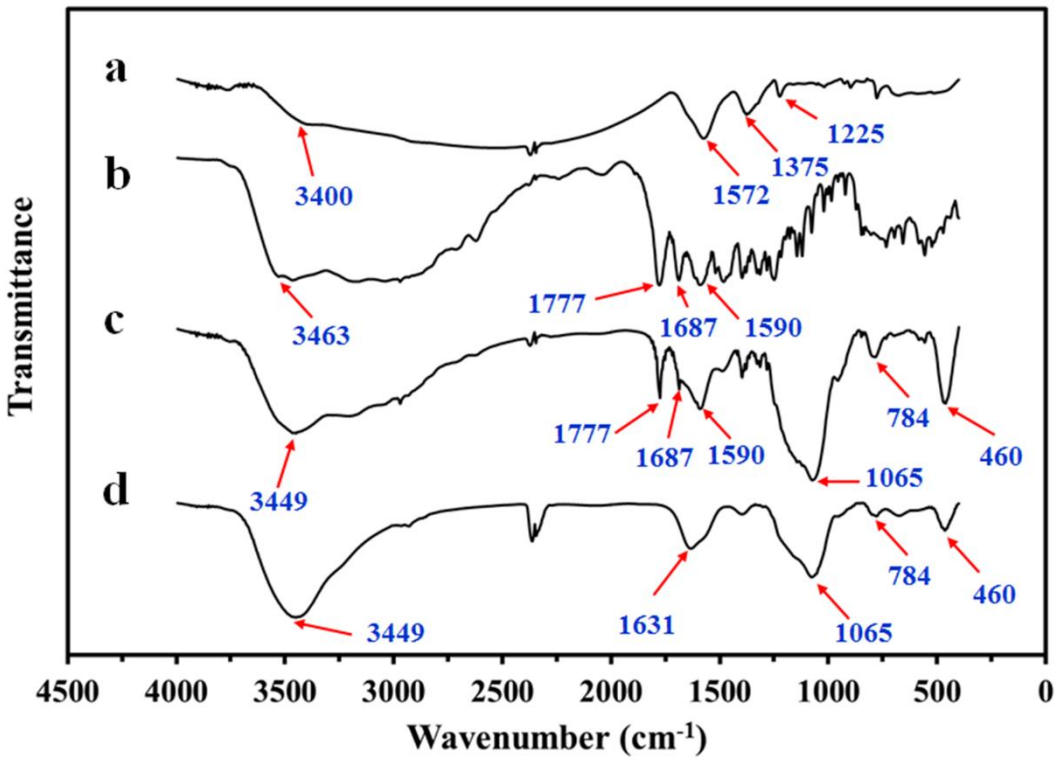

a
b
c
d
Transmittance
3400
1572
1375
1225
3463
1777
1687
1590
3449
1777
1687
1590
784
460
1065
3449
1631
1065
784
460
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Wavenumber (cm-1)

Fig. 4

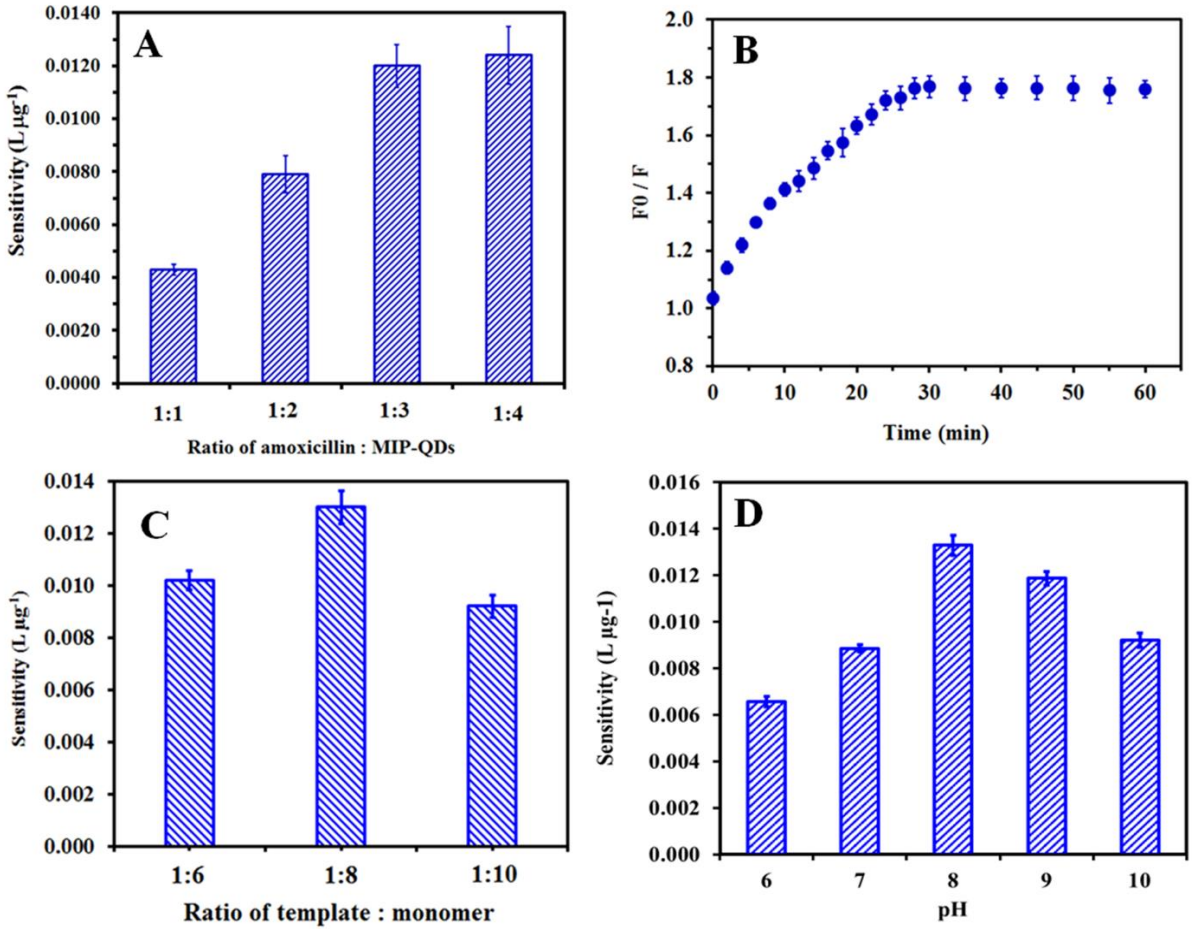
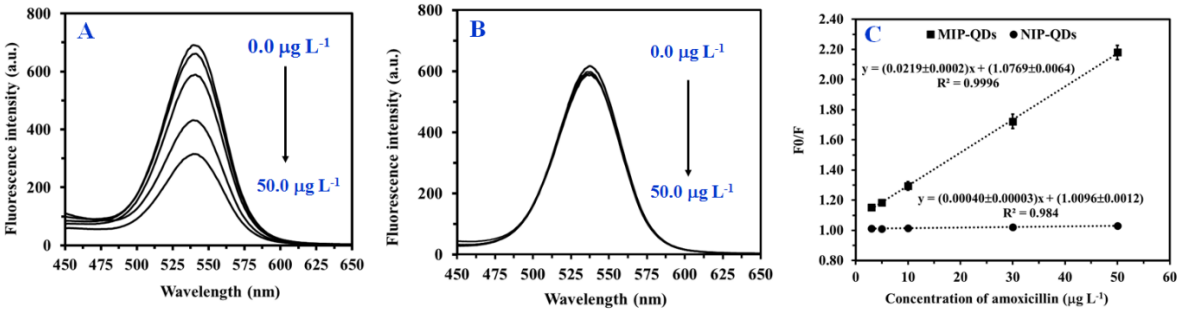

A
Sensitivity (L μg-1)
0.0140
0.0120
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
1:1
1:2
1:3
1:4
Ratio of amoxicillin : MIP-QDs
B
F0 / F
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
10
20
30
40
50
60
Time (min)
C
Sensitivity (L μg-1)
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
1:6
1:8
1:10
Ratio of template : monomer
D
Sensitivity (L μg-1)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
6
7
8
9
10
pH

Fig. 5


A
0.0 µg L-1
50.0 µg L-1
Fluorescence intensity (a.u.)
Wavelength (nm)
B
0.0 µg L-1
50.0 µg L-1
Fluorescence intensity (a.u.)
Wavelength (nm)
C
MIP-QDs
NIP-QDs
y = (0.0219±0.0002)x + (1.0769±0.0064)
R² = 0.9996
y = (0.00040±0.00003)x + (1.0096±0.0012)
R² = 0.984
F0/F
Concentration of amoxicillin (µg L-1)

F

Fig. 6

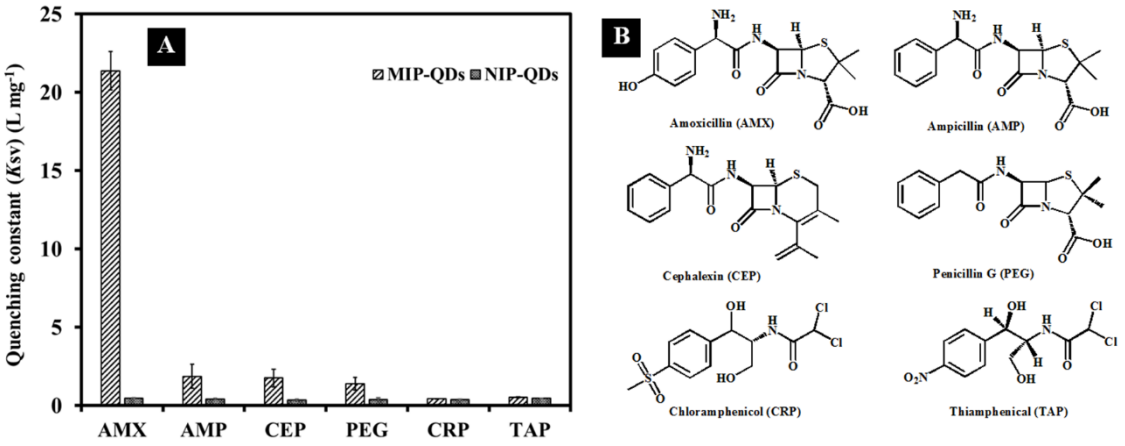

A
MIP-QDs
NIP-QDs
Quenching constant (Ksv) (L mg-1)
25
20
15
10
5
0
AMX
AMP
CEP
PEG
CRP
TAP
B
Amoxicillin (AMX)
Ampicillin (AMP)
Cephalexin (CEP)
Penicillin G (PEG)
Chloramphenicol (CRP)
Thiamphenical (TAP)

Fig. 7

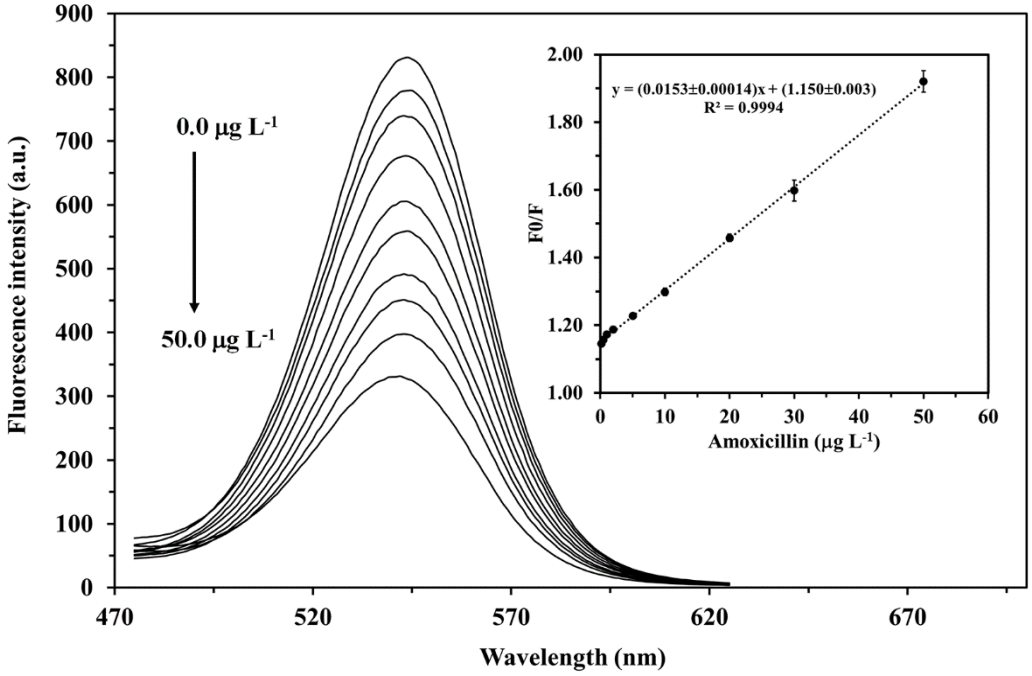

Fluorescence intensity (a.u.)
Wavelength (nm)
0.0 µg L-1
50.0 µg L-1
F0/F
Amoxicillin (µg L-1)
y = (0.0153±0.00014)x + (1.150±0.003)
R² = 0.9994

Fig. 8

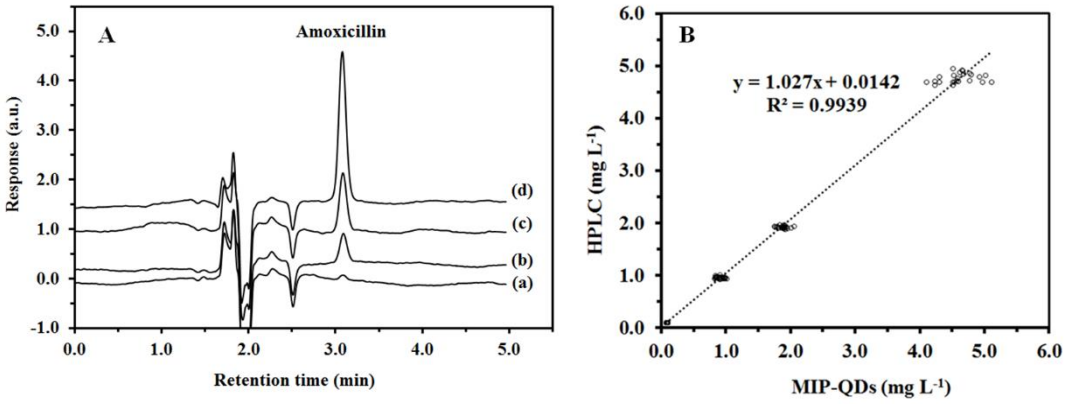

A
Amoxicillin
Response (a.u.)
Retention time (min)
(d)
(c)
(b)
(a)
B
y = 1.027x + 0.0142
R² = 0.9939
HPLC (mg L⁻¹)
MIP-QDs (mg L⁻¹)

Fig. 9